ORIGINAL CONTRIBUTION

Influence of methoprene and dietary protein on maturation and sexual performance of sterile *Anastrepha ludens* (Diptera:Tephritidae)

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Abstract

The success of the sterile insect technique (SIT) requires the release of males that can compete with wild males. Elevated juvenile hormone (JH) levels and hydrolysed yeast-enriched adult diets improve the sexual attractiveness and competitiveness of sterile male *Anastrepha ludens* (Loew; Mexican fruit fly), used in large-scale SIT programmes in Mexico and Texas. We applied methoprene (a JH analogue) to *A. ludens* using two methods, topically on individual sterile adults and mass- immersion of pupae after irradiation. Hydrolysed yeast was either used dry or in agar blocks. Laboratory and field cage experiments were conducted to compare male maturation and sexual performance when males exposed to four different treatments competed for females: (M^+P^+) application of methoprene and sugar plus hydrolysed yeast as adult food; (M^+P^-) application of methoprene and sugar only as adult food; (M^-P^+) no application of methoprene and sugar plus hydrolysed yeast as adult food; and (M^-P^-) no application of methoprene and sugar only as adult food. Methoprene and hydrolysed yeast accelerated the male sexual maturation from 9 to 5 days after the emergence. Combined application of methoprene and hydrolysed yeast had a significant effect on increased male sexual performance in both laboratory and field cage tests. Methoprene application also accelerates female maturation, although less than in males. The substantial improvements in male sexual performance and sexual maturation that can be obtained by incorporating the use of JH analogue and hydrolysed protein, and the development of techniques for their practical application on a large scale, can contribute to a more cost-effective deployment of sterile male Mexican fruit flies in SIT programmes.

Introduction

Males of polyphagous fruit flies (Diptera: Tephritidae), such as several pestiferous *Anastrepha* spp., emit chemical, acoustic and visual sexual signals and often aggregate in leks to attract females (Prokopy 1980; Burk 1981; Sivinski and Burk 1989). Receptive females arrive at these aggregations to choose mates, and the variance in male reproductive success is typically high, i.e. a relatively small proportion of the males obtain the majority of copulations (Shelly and Whittier 1997; Field et al. 2002). Differences in sexual signal performance and activity play a role in generating this variance. Because the success of the sterile insect technique (SIT) requires males that can compete in these leks and be identified by females as

suitable mates (Knipling 1955), it is important to fully understand the target-pest's sexual maturation and mating behaviour and to incorporate the best possible sexual qualities into mass-reared sterile insects (Hendrichs et al. 2002).

The Mexican fruit fly, *Anastrepha ludens* (Loew), is one such lekking fly and the target of some large-scale SIT programmes (Dowell et al. 2005). It is a polyphagous species distributed from southern Texas through much of Central America, whose original hosts are species of Rutaceae, *Casimiroa greggii* (S. Wats.) and *Casimiroa edulis* La Llave & Lex. (Aluja et al. 2009). *Anastrepha ludens* is the most important indigenous fruit fly pest of citrus in the New World, particularly of *Citrus paradisi* Macfad. (grapefruit; Rutacaea). It also infests mangoes (Anacardiaceae), peaches (Rosaceae) and peppers (Solanaceae; Norrbom 2004; Thomas 2003, 2004; Birke et al. 2006).

In some *Anastrepha* spp., absorption of juvenile hormones (JH) analogues like methoprene and consumption of dietary-hydrolysed yeast (source of protein) during the adult pre-sexual maturation periods accelerate male tephritids development and enhance sexual success, perhaps through increased pheromone production (Teal et al. 2000; Teal and Gómez-Simuta 2002a). The advantages of greater sexual ability are self-evident, but SIT programmes also benefit from more rapid sterile male sexual maturation. Pre-release insect holding space can be reduced; in addition, maturing sterile males can survey immediately for mates increasing the effectiveness of the SIT (FAO/ IAEA 2007). In fruit fly species with long adult pre-copulatory periods, like *Anastrepha* spp. (Aluja 1994), early maturation is therefore particularly important.

Because maturation, territorial defence and courtship may depend on adult-acquired nutritional resources in many tephritids, accelerated maturity owing to hormone manipulation might leave less time for flies to feed and store reserves. Thus, additional adult-dietary protein could be particularly important when exogenous hormones are used to accelerate development (Pereira et al. 2010a). Sexual performance is also improved by providing hydrolysed yeast in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.; Blay and Yuval 1997; Yuval et al. 2007; Barry et al. 2007; Gavriel et al. 2010) and *Bactrocera tryoni* (Perez-Staples et al. 2007), as well as several species of *Anastrepha*: *A. suspensa* (Loew; Teal et al. 2007; Pereira et al. 2009, 2010a); *A. fraterculus* (Wied.; Segura et al. 2009); and *A. ludens* (Loew; Gómez and Teal 2010, Gómez et al. in review).

In this study, we determined the effects of exposure to a JH analogue (methoprene), adult-dietary-hydrolysed yeast and their interactions on sexual maturation and performance of male *A. ludens* and on sexual maturation in females. Specifically, we measured (i) time to reach sexual competence and (ii) male mating success measured by copulation. Methoprene was applied either (i) topically to individual adults (Pereira et al. 2009) or (ii) through pupal immersion in water or acetone solutions. The two techniques were compared to further development of practical large-scale methods of methoprene treatment. Similarly, hydrolysed yeast was tested dry, mixed with sugar or incorporated into agar blocks. The implications of our results for the handling of sterile males in Mexican fruit fly SIT programmes are discussed.

Material and Methods

Insects

Mexican fruit flies used in the study were obtained from the USDA/APHIS/PPQ mass-rearing facility in Mission, TX, and were reared according to the mass-rearing facility protocol (Stevens 1991). All flies used in experiments were irradiated as pupae 24–48 h before emergence in a Cs^{137} irradiator (Husman 521; Isomedix, Grand Prairie, TX) with a dose of 70 Gy.

After emergence, adults were separated by sex and maintained under a photoperiod of 14L:10D (light from 0600 to 2000 h) with light intensity of 450 ± 50 lux, temperature of $25 \pm 1°C$ and relative humidity of $70 \pm 5\%$. All laboratory experiments were conducted in this same room under the same environmental conditions.

Methoprene applications

Two methods to apply methoprene were compared. For the individual adult topical application, methoprene was applied topically in the first 24 h after adult emergence at a rate of 5 μg in 1 μl acetone solution per male. In M^- treatments, 1 μl of acetone was applied, to serve as a control (Pereira et al. 2009). Males were immobilized in a net bag [as in standard marking techniques, FAO/IAEA/USDA (2003)] and the solution applied via pipette through the net onto the dorsal surface of the thorax. No anaesthesia was used to immobilize the flies. Two different net bags and pipette tips were used (one for M^+ treatments and another for M^- treatments) to prevent methoprene contamination. Males from each treatment were maintained in separate

$30 \times 30 \times 40$ cm acrylic cages with the type of food assigned for each treatment.

For the pupae immersion, immediately after irradiation, pupae were immersed for 5 min in a 0.005% solution of methoprene. The solution was made by dissolving 100 ul of an emulsifiable concentrate containing 5% methoprene (Wellmark International, Dallas, TX) in 1 l of water or acetone.

To evaluate the impact of the immersion in water and acetone, we measure the emergence and fliers according the FAO/IAEA/USDA (2003) quality control manual. Five tubes (plus an empty tube) of 100 irradiated pupae each and representing the treatments listed below and were subsequently tested over three consecutive days:

A. control: no immersion

B. immersion in acetone solution with 0.005% methoprene.

C. immersion in water solution with 0.005% methoprene

D. immersion in acetone (control of B)

E. immersion in water (control of C)

Owing to the results of this methoprene application experiment, which indicated a negative impact of immersion in water-methoprene solution (C) on the percentage of emergence and on flight ability, we conducted subsequent experiments only with pupae immersed in methoprene–acetone solution (B).

Dietary protein

Two methods of presenting hydrolysed yeast to the adult diet were used for the different studies. One method consisted of hydrolysed yeast added to sugar in a proportion of four parts of sugar and one part of hydrolysed yeast (4 : 1, 20% hydrolysed yeast). In the second, 5% hydrolysed yeast was incorporated immediately before solidification of agar in the preparation of the agar blocks that are provided to sterile males in fly emergence and release facilities (FAO/IAEA 2007). In P$^-$ controls only sugar or sugar in agar blocks were supplied to the flies (USDA/APHIS 2009), and in all treatments, water was provided *ad libitum*. The first method was used when the methoprene was applied topically and the second method when the methoprene was applied by immersion.

Treatments

Sterile male *A. ludens* subjected to the treatments below were compared in terms of sexual maturation and mating performance:

M$^+$P$^+$: application of the JH analogue, methoprene (M) and sugar and hydrolysed yeast (source of protein and other nutrients) as adult food (P);

M$^+$P$^-$: methoprene application and sugar only as adult food;

M$^-$P$^+$: no methoprene application and sugar and hydrolysed yeast as adult food;

M$^-$P$^-$: no methoprene application and sugar only as adult food.

After emergence, flies were separated by sex (and metoprene treated in the case of individual adult topical application) and maintained in separate $30 \times 30 \times 40$ cm acrylic cages with the type of food assigned for each treatment. Sexual maturation and mating success in laboratory and field cage tests were evaluated for both methoprene application methods.

Sexual maturation

Sterile males from the age of 2 days until 10 days old and treated with the four combinations of methoprene exposure (both topically as adults and by immersion as pupae) and protein availability (hydrolysed yeast provided or not) were exposed to irradiated females (13–15 days old) with access to sugar and hydrolysed yeast (4 : 1). The proportions of males mating indicated sexual maturation. Daily, at 1600 h, 10 males from the assigned treatments were released into $30 \times 30 \times 40$ cm cages (four cages per treatment per age). Fifteen minutes later, 20 mature females were released inside each cage. Insects were surveyed continuously and pairs registered and removed as soon as they started to mate. Observations lasted until 2000 h (end of the photoperiod in laboratory), covering the late afternoon period of sexual activity in *A. ludens* (Aluja et al. 2000). No food or water was supplied to the flies during the experimental period.

Mating success in laboratory

Four 12- to 13-days-old males (one per treatment) were released simultaneously into $30 \times 30 \times 40$ cm cages at 1600 h. These were marked in the morning with a dot of water-based paint (different colour per treatment and rotated among treatments) on the dorsal surface of the thorax (FAO/IAEA/USDA 2003). Fifteen minutes later, one 13- to 15-days-old, sexually mature virgin female was released into each cage and observed until 2000 h. No food or water was supplied during the experimental period. When mating occurred, the identity

of the successful male was noted. A total of 64 cages were observed (16 cages per day during four consecutive days) for each methoprene application method.

Mating success in field cages

The experiment was conducted under outdoor conditions in $2.9 \times 2.9 \times 2$ m field cages in which two 1.5-m-high-potted grapefruit trees (*C. paradisi*) served as a substrate for calling males and sexual interactions. Procedures from the quality control manual (FAO/IAEA/USDA 2003) were followed with some adaptation described later. In this experiment, four cages were observed daily over 4 days for a total of 16 replications per each methoprene application method. Each type of methoprene application was tested separately. One hundred virgin males (25 per treatment), 12–13 days old and colour marked as above, were released simultaneously at 1600 h. Thirty minutes later, 50 virgin sexual mature females, 13–15 days old, were added into the field cage. The experiment ran until completely dark (1900 h, note that sunset occured at ca. 1830 h during the experiment) to coincide with *A. ludens*'s sexual activity period (Aluja et al. 2000). During these observation periods, temperature, relative humidity and light intensity were measured every 30 min. Mating pairs were removed and the type of male mating recorded.

Effect of methoprene on female and male maturation

We compared time until sexual maturation by noting the proportions of females and males mating on consecutive days of age. Pupae were exposed after irradiation to methoprene in both water and acetone solutions. Three treatments [M^+(water)P^+, M^+(acetone)P^+ and M^-P^-] were compared in the three different contexts later:
- males (3, 4, 5…,12 days) with mature females of 14–17 days (10 males and 20 females)
- females (3,4,5…,12 days) with mature males of 12–15 days (10 females and 20 males)
- males (3,4,5…,12 days) with females of the same age (10 males and 10 females).

There were four repetitions a day (per age) per procedure.

Statistical analyses

Data were analysed by two-way analysis of variance (ANOVA) with methoprene, hydrolysed yeast and their interaction serving as independent class variables. When appropriate, these analyses were followed by single-variable ANOVAs and Tukey's mean separation tests (P = 0.05) to identify significantly different means (Ott and Longnecker 2001). Statistical analyses were performed using R software (version 2.1.0, http://www.r-project.org).

Results

The percentage of emergence and of fliers was significantly higher when irradiated pupae were immersed in a methoprene and acetone solution than in methoprene and water (percentage emergence: $F_{4, 11} = 5.07$, P = 0.023; and percentage fliers: $F_{4, 11} = 9.23$, P = 0.011; fig. 1). The water solution turned milky and sticky which appeared to adhere to the puparia, impeded emergence and decreased fliers.

Sexual maturation

Both methoprene and hydrolysed yeast accelerated male sexual maturation either when methoprene was applied topically to adults (fig. 2a) or by pupal immersion (fig. 2b). Assuming that a frequency of 80% of males mating implies full cohort maturation, then the individual topical methoprene application method (fig. 2a) yielded the following: (i) the incorporation of hydrolysed yeast into adult diets (P^+) accelerates male maturation, both in methoprene-
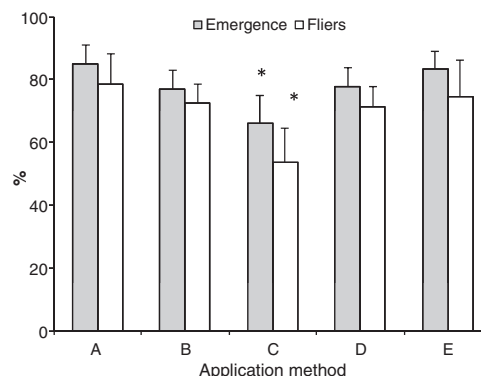
Fig. 1 Percentage emergence and fliers (mean ± SD) of *Anastrepha ludens* according the different methods of treating irradiated pupae (a) control, no immersion; (b) immersion in acetone solution with 0.005% methoprene; (c) Immersion in water solution with 0.005% methoprene; (d) Immersion in acetone (control of B); (e) Immersion in water (control of C). Data were obtained from 15 replications. (*) represent significant differences (for % emergence and % fliers; Tukey's test, P = 0.05).
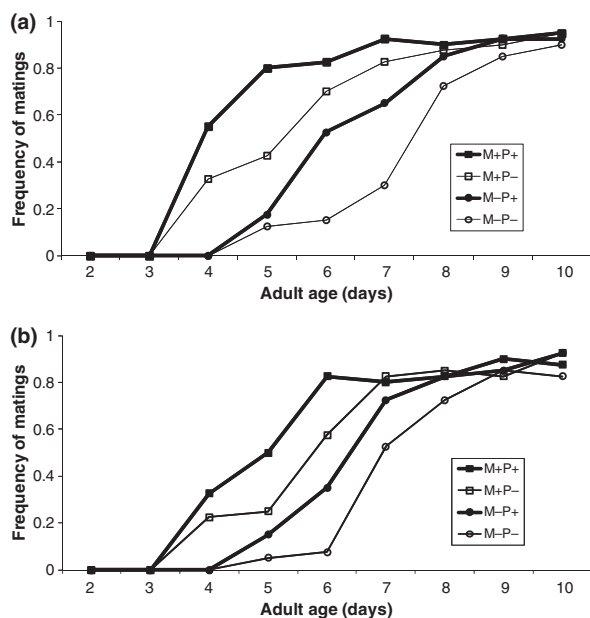
Fig. 2 Frequency of matings in laboratory of male *Anastrepha ludens* at different adult ages, when treated or not with methoprene (M$^+$/M$^-$) and fed or not with protein (P$^+$/P$^-$). Individual adult topical application (a) or pupae immersion (b). Data were obtained from the four replications per treatment per age in both methods.
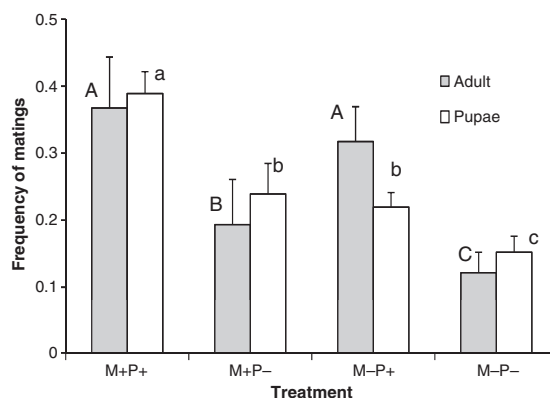
Fig. 3 Frequency of matings in laboratory of male *Anastrepha ludens*, when treated or not with methoprene (M$^+$/M$^-$) and fed or not with protein (P$^+$/P$^-$) (mean ± SD). Methoprene was topically applied to adults individually (adults) or to mature pupae immerged in a methoprene solution (pupae). Data were obtained from the four replications of 16 laboratory cages with five flies each (one female and four males, one from each treatment) for each method. Different capital and lowercase letters represent significant differences between treatments for methoprene application method (adult or pupae), respectively (Tukey's test, P = 0.05).

treated (from 7 to 5 days) and methoprene-untreated males (from 9 to 8 days); (ii) methoprene application (M$^+$) accelerates male maturation, both in protein-fed (from 8 to 5 days) and protein-deprived males (from 9 to 7 days); and (iii) the incorporation of protein and application of methoprene (M$^+$P$^+$) leads to maturation in 4 days (at 5 days of age) compared with the standard procedure (methoprene-untreated and protein-deprived P$^-$M$^-$).

For the pupal immersion method of methoprene exposure (fig. 2b), (i) the incorporation of hydrolysed yeast into adult diets (P$^+$) accelerated male maturation, in both methoprene-treated (from 7 to 6 days) and methoprene-untreated males (from 9 to 8 days); (ii) methoprene application (M$^+$) accelerates male maturation, in both protein-fed (from 8 to 6 days) and protein-deprived males (from 9 to 7 days); and (iii) the incorporation of hydrolysed yeast and application of methoprene (P$^+$M$^+$) accelerates maturation by 3 days (to 6 days) compared to methoprene-untreated and protein-deprived males (P$^-$M$^-$).

Mating success in laboratory

A total of 128 cages containing P$^+$M$^+$, P$^-$M$^-$, P$^+$M$^-$ and P$^-$M$^+$ males (64 for each of the two methoprene application methods) were observed to determine treatment effects on mating propensity. Regardless of application method, there were significant positive effects for methoprene application protein supply and significant interactions between methoprene and protein ($F_{1, 12} = 12.07$, P = 0.003 for individual adult topical application and $F_{1, 12} = 21.32$, P < 0.001 for pupal immersion). Therefore, data of all four treatments were analysed using one-way ANOVAs for both methoprene application methods ($F_{3, 12} = 43.09$, P < 0.001 for individual adult topical application and $F_{3, 12} = 45.12$, P < 0.001 for pupal immersion) followed by the Tukey's tests (fig. 3).

With the topical adult application method, 57 successful matings were recorded in the laboratory so that 89% of all females mated. Of these matings, 37% were performed by M$^+$P$^+$ males, which was statistically similar to the numbers of M$^-$P$^+$ males that mated (32%), while M$^+$P$^-$ (19%) and M$^-$P$^-$ (12%) males mated significantly less than the protein-fed males (fig. 3).

Males derived from immersed pupae performed 59 matings so that 92% of all females mated. Of the total matings, 39% were performed by M$^+$P$^+$ males, who performed significantly better than M$^+$P$^-$ (24%) and M$^-$P$^+$ (22%) males. M$^-$P$^-$ males obtained the fewest copulations, obtaining just 15% of the matings (fig. 3).

Mating success in field cages

The abiotic conditions during the 4 days of the field cage experiment were similar, with temperatures from 23°C to 32°C and from 24°C to 33°C, respectively, for the individual adult topical application and pupae immersion methods. The RH varied from 58% to 84% and from 52% to 78%, respectively. Light intensity dropped from close to 12 000 lux at 1600 h to values below 200 lux at 1830 h in both cases. No matings occured before the light intensity dropped below 2000 lux (ca. 1 h before sunset), while they occurred between this light intensity and completely dark.

A total of 32 cages (16 each containing males exposed to one of the two methoprene application methods) were observed to compare the sexual abilities of P^+M^+, P^-M^-, P^+M^- and P^-M^+ males. For both methods, there were significant positive effects for methoprene application protein supply and significant interactions between methoprene application and protein supply ($F_{1, 12} = 22.12$, $P < 0.001$ for individual adult topical application and $F_{1, 12} = 24.01$, $P < 0.001$ for pupal immersion). Therefore, data were analysed using one-way ANOVAs to compare all four treatments for both methoprene application methods ($F_{3, 12} = 31.10$, $P < 0.001$ for individual adult topical application and $F_{3, 12} = 26.11$, $P < 0.001$ for pupal immersion) followed by Tukey's tests (fig. 4).

Males exposed to methoprene through individual adult topical application participated in 460 matings, so that 58% of all females mated. The M^+P^+ males with 170 matings (37%) performed significantly better than M^+P^- and M^-P^- males, who obtained 21% and 14% of the matings, respectively, but not significantly better than M^-P^+ males with 28% of matings (fig. 4). Males exposed to methoprene by pupal immersion mated in similar proportions. A total of 396 males and 50% of all females copulated. The M^+P^+ males with 152 matings (39%) performed significantly better than M^+P^- and M^-P^- males, who obtaining 20% and 14% of the matings, respectively, but not significantly better than M^-P^+ males with 27% of matings (fig. 4).

Effect of methoprene on female maturation

Even without exposure as pupae to methoprene, females matured 2 days later (10 days) compared with males (8 days; fig. 5). When pupae were immersed in a methoprene solution (either water or acetone) maturation for both sexes was accelerated by 2–3 days (5 days in males, 7–8 days in females; fig. 5).

Discussion

Male and female *A. ludens* reached sexual maturity more rapidly following topical methoprene exposure. In the laboratory, males treated with methoprene and not provided hydrolysed yeast were more likely to mate than males that had neither protein nor methoprene, while in field cages; there was no
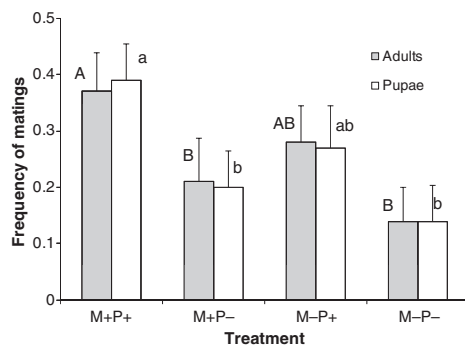

Fig. 4 Frequency of matings in field cages of male *Anastrepha ludens*, when treated or not with methoprene (M^+/M^-) and fed or not with protein (P^+/P^-) (mean ± SD). Methoprene was topically applied to adults individually (adults) or to mature pupae immerged in methoprene solution (pupae). Data were obtained from 16 field cages with 150 flies (50 females and 25 males from each treatment) for each method. Different capital and lowercase letters represent significant differences between treatments for methoprene application method (adult or pupae), respectively (Tukey's test, P = 0.05).
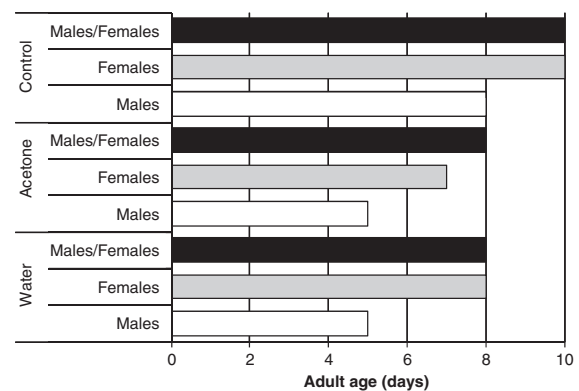

Fig. 5 Sexual maturation measured by ability to mate (first mating occours) of both sexes of *Anastrepha ludens* when mature pupae were immersed in methoprene solution (both in water or acetone) in comparison with the untreated control: Males/Females (when crosses involved both sexes with the same test age); Males (when maturing males were crossed with mature females) and Females (when maturing females were crossed with mature males).

significant effect of methoprene alone on mating success. The addition of hydrolysed yeast to the adult diet accelerated sexual maturation in males, albeit to a lesser degree than methoprene alone and also substantially improved the likelihood of a male copulating. When combined, a synergistic effect of (M^+P^+) was observed.

Overall, methoprene had a similar effect whether applied individually to newly emerged adults or by mass immersion of pupae, suggesting a practical means to employ methoprene following mass rearing and sterilization. Also, hydrolysed yeast could be efficiently provided either as a dry mixture or incorporated into the commonly used agar blocks (FAO/IAEA, 2007). Again, this generates options for large-scale applications, particularly in view that the incorporation of hydrolysed yeast can result in sticky agar blocks, causing high fly mortality in fly emergence and release facilities.

Our present *A. ludens* results differ, to one degree or another, from previous studies of *A. ludens* and other *Anastrepha* spp. Sexual male maturation was advanced by the methoprene application and to a lesser extent by the protein supply. While topical methoprene applications improved sexual performance only in the laboratory experiment and not to the same extent by the dietary protein, this was not the case in the closely related *A. suspensa*. In this species, methoprene and dietary protein increased sexual competency to a similar extent. Their combination was additive and yielded a significant improvement in sexual success (Pereira et al. 2009, 2010b). Similar results have also been found in *A. fraterculus* (Segura et al. 2009). Male *A. serpentina* provided methoprene in a sugar diet 'called' (a combined acoustic and chemical sexual signal) more often, but this was not the case in *A. obliqua* or *A. striata* (Aluja et al. 2009).

Acceleration of female maturation with JH analogues was previously studied by Segura et al. (2009), who report a differential response to methoprene application in male and female *A. fraterculus* that acts as a physiological sexing system. However, unlike Segura et al. (2009), who found no effect of the methoprene in female *A. fraterculus,* our results with *A. ludens* indicate some effect of the methoprene in female *A. ludens* and thus only a reduced differential response between sexes. However, their experimental design and objectives were quite different from ours.

Although there was a significant interaction between protein and methoprene in our data, the relationship of nutritional status to methoprene is more complex than suggested by our experiments

alone. Aluja et al. (2009) showed that changes in the sexual behaviour and maturation rates of adult male *A. ludens* following consumption of dietary methoprene depended on their developmental history. In those flies obtained from *C. greggii*, the putative ancestral host in which larvae feed on seeds, methoprene accelerated the first appearance of male calling behaviour and also the number of copulations obtained. But methoprene had no effect when flies were collected from *C. paradisi*, an exotic host in which larvae feed on pulp. In the absence of methoprene, there was a substantial host, presumably nutritional effect, with males from *C. paradisi* calling and mating nearly twice as often as those from *C. greggii*. A plausible interpretation of Aluja et al. (2009) that is somewhat consistent with our present results is that the effects of methoprene were masked by the physiological advantages enjoyed by insects originating from a nutritionally enriched host background.

In terms of sexual maturation, methoprene accelerated both *A. ludens* and *A. suspensa* at similar rates (Pereira et al. 2009). Aluja et al. (2009) report a different effect of methoprene application in *A. ludens* when larvae developed in different hosts (larval diets).

There are clearly important relationships between nutritional status (adult diet and host background) and JH activity in *Anastrepha* spp. Readily available sugar is known to be a basic requirement for survival and all activities in male tephritids (Landolt and Sivinski 1992; Teal et al. 2004). Providing in addition protein, however, has had more mixed results. Incorporation of hydrolysed yeast in the adult diet has already been shown to improve male sexual success in tephritids such as *C. capitata* (Blay and Yuval 1997; Yuval et al. 1998; Kaspi et al. 2000; Gavriel et al. 2010), *A. obliqua, A. serpentina, A. striata* (Aluja et al. 2001) and *A. suspensa* (Landolt and Sivinski 1992; Pereira et al. 2010a). Taylor and Yuval (1999) reported that female *C. capitata* store more sperm when they copulate with protein-fed males, which reduces subsequent female remating. In contrast to the present results, Aluja et al. (2001) found no effect of providing protein on male *A. ludens* sexual success. However, the same group (Aluja et al. 2008) reports an increase in the mating success of protein-fed males of *A. ludens* and *A. striata*, regardless of the size of the males tested. Thus, the effects of protein-addition may be conditional, as apparently are those of methoprene.

We suggest that the relationship of artificial larval diet components and exposure to both adult-dietary

protein and methoprene be further explored. There may be significant opportunities to 'fine-tune' sterile male, and female, performance by manipulating all three variables. Regardless of physiological details and the possibility of future improvements, the present results already clearly suggest means to improve *A. ludens* sterile male quality. The acceleration of male maturation up to 4 days with methoprene treatments, and to a lesser degree by incorporating hydrolysed yeast in the pre-release diet, could reduce the time of handling flies in emergence facilities, lessen the space required for adult-storage prior to release and allow the release of sterile flies closer to sexual maturity, thus avoiding inevitable pre-maturation mortality in the field. All of this contributes to cost reductions and increased effectiveness. Ultimately, SIT depends on the release of competitive sterile males (Knipling 1955). Increased male signalling and pheromone production owing to hormonal and nutritional supplements, while not physiologically well understood (Teal and Gómez-Simuta 2002b), leads to both significantly greater lifetime sexual success in sterile males (Teal et al. 2000; Pereira et al. 2010a).

Acknowledgements

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